

RECENT RESEARCH ON THE WESTERN CORN ROOTWORM

Behaviour and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte)

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- Abstract**
- 1 The western corn rootworm (WCR) is a historic pest with a legacy of resistance and behavioural plasticity. Its behaviour and nutritional ecology are important to rootworm management. The success of the most effective and environmentally benign rootworm management method, annual crop rotation, was based on an understanding of rootworm behaviour and host–plant relationships. Enthusiastic adoption of crop rotation, provided excellent rootworm management, but also selected for behavioural resistance to this cultural control.
 - 2 Though well-studied, significant gaps in WCR biology remain. Understanding the topics reviewed here (mating behaviour, nutritional ecology, larval and adult movement, oviposition, alternate host use, and chemical ecology) is a starting point for adapting integrated pest management and insect resistance management (IRM) to an expanding WCR threat. A presentation of significant questions and areas in need of further study follow each topic.
 - 3 The expansion of WCR populations into Europe exposes this pest to new environmental and regulatory conditions that may influence its behaviour and ecology. Reviewing the state of current knowledge provides a starting point of reference for researchers and pest management decision-makers in North America and Europe.
 - 4 The trend toward increasing adoption of transgenic maize will place an increasing premium on understanding WCR behaviour. IRM plans designed to promote sustainable deployment of transgenic hybrids are grounded on assumptions about WCR movement, mating and ovipositional behaviour. Preserving the utility of new and old management options will continue to depend on a thorough understanding of WCR biology, even as the ecological circumstances and geography of WCR problems become more complex.

Keywords Chrysomelidae, Coleoptera, crop rotation resistance, maize, mating, movement, nutritional ecology, oviposition, semiochemicals, *Zea mays*.

Introduction

The western corn rootworm (WCR) *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) was first collected in the U.S.A. in 1867 (LeConte, 1868) and recognized

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as a pest in 1909 (Gillette, 1912). Today, it is the most important pest of maize (*Zea mays* L.) in the U.S. Corn Belt. Annual U.S. pest management costs and maize production losses attributable to corn rootworms [chiefly WCR and the related northern corn rootworm (NCR) *Diabrotica barberi* Smith and Lawrence] probably exceed US \$1 billion (Metcalf, 1986; Rice, 2004). After emergence from overwintering eggs, larvae feed on maize roots. Apart from maize, larval development to adult is also possible on a number non-maize grasses (Oyediran *et al.*, 2004; Wilson & Hibbard, 2004). Larval feeding interferes with water and nutrient

uptake, facilitates pathogen entry, and reduces the ability of roots to hold maize plants upright (and therefore to withstand lodging) (Levine & Oloumi-Sadeghi, 1991). During the single generation each year, adults are present in maize fields from late June until the autumn frost.

A strong ovipositional fidelity to maize fields combined with an inability to complete development on most other crops made the NCR and WCR ideal candidates for control via annual crop rotation for more than 100 years (Spencer & Levine, 2008). Annually alternating maize planting with that of a nonhost plant (i.e. soybean) would result in the death of larvae emerging from eggs deposited in maize fields. Writing in the report of the State Entomologist of Illinois, Stephen A. Forbes, asserted that crop rotation was such a plainly obvious solution to the problem of corn rootworm control that 'no special comment was required' to explain his reasoning (Forbes, 1883). Forbes' biologically-based recommendation was correct and remains an effective method to manage rootworm injury across much of the U.S. Corn Belt, with some notable exceptions (Levine *et al.*, 2002). In fields where maize is planted in consecutive years (continuous maize), rootworm management was primarily accomplished by planting-time soil-insecticide application or foliar sprays (Levine & Oloumi-Sadeghi, 1991). Subsequent to 2003, rootworm-resistant transgenic maize hybrids (with their accompanying neonicotinoid seed treatment) have been widely adopted by farmers (Rice, 2004; Gray & Steffey, 2007; USDA-ERS, 2007).

The success of crop rotation highlights the importance of understanding pest biology to improve pest management. Yet, even within an integrated pest management (IPM) framework, broad adoption of annual crop rotation ultimately selected for populations of NCR by the 1960s and WCR by the 1990s with resistance to what seemed like an unbeatable cultural control (Spencer & Levine, 2008). A desire to avoid rapid resistance to rootworm-resistant transgenic maize hybrids expressing insecticidal proteins from the soil microbe, *Bacillus thuringiensis* (*Bt*) (Vaughn *et al.*, 2005), has once again focused interest on WCR ecology and behaviour.

To preserve the continued utility of *Bt* technology, the U.S. EPA mandates implementation of an insect resistance management (IRM) plan for all insect-resistant *Bt* crops (US-EPA, 2001). The rootworm IRM plan includes the use of nontransgenic maize refuges within or adjacent to fields where a rootworm-resistant hybrid is grown. Refuges are sites where susceptible genotypes can survive. It is expected that *Bt*-susceptible refuge males will disperse throughout the transgenic crop and out-compete the relatively less abundant, but potentially-resistant, transgenic-field males for mating opportunities. The refuge strategy for NCR and WCR is grounded on literature-based assumptions about rootworm biology, ecology, and the genetics of resistance. Perhaps more than at any previous time, the sustainability of a management tool depends on a thorough understanding of pest behaviour, ecology and evolution.

The recent (and probably continuing) introduction of WCR into Europe (Miller *et al.*, 2005) has also generated new interest in understanding WCR behaviour. For scientists unfamiliar with WCR, a thorough review of WCR biology in the home range, informed by the latest analyses of WCR behaviour in the introduced range will improve monitoring and management.

Understanding the established behavioural repertoire of a pest is key to managing it under new circumstances. It is in that spirit that this review of WCR behaviour and ecology is undertaken.

Courtship and mating behaviour

The WCR is a protandrous species; the first adult males emerge approximately 5 days before females (male larval development is faster than that of females) (Branson, 1987). Despite protandry, Quiring and Timmins (1990), note that 97.8% of the male emergence period overlaps with that of females. After males emerge, approximately 5–7 days of post-emergence development are required for 80% of males to reach sexual maturity (characterized by responsiveness to female sex pheromone) (Guss, 1976). WCR females are sexually mature upon adult emergence (Hammack, 1995); many mate within hours of emergence (Ball, 1957; Hill, 1975; Lew & Ball, 1979).

Sex pheromone

Cates (1968) first suggested that a sex pheromone was involved in WCR reproduction. Ball and Chaudhury (1973) first published evidence of a female-extractable sex pheromone by demonstrating elevated male WCR trap catch on female-extract baited sticky traps in the field. The major active component of the WCR sex pheromone is 8R-methyl-2R-decenyl-propanoate (Guss *et al.*, 1982). The WCR sex pheromone was the first to be identified and synthesized from a chrysomelid (Guss *et al.*, 1982), although the first evidence of pheromone use by a chrysomelid came from the banded cucumber beetle *Diabrotica baleata* LeConte (Cuthbert & Reid, 1964). The same molecule is also the active component of the sex pheromone of the Mexican corn rootworm *Diabrotica virgifera zea* (MCR); the two closely-related subspecies (Krysan *et al.*, 1980) have nearly identical response profiles to the pheromone (Guss *et al.*, 1984). The NCR also uses 8R-methyl-2R-decenyl-propanoate (8R,2R) as its sex pheromone (Guss *et al.*, 1982; Dobson & Teal, 1987). Wilkin *et al.* (1986) performed electroantennogram (EAG) bioassays of gas-liquid chromatographic fractions of volatiles collected from virgin female WCR, southern corn rootworm *Diabrotica undecimpunctata howardi* Barber (SCR), and *Diabrotica longicornis*. One active fraction was found for each species, corresponding to structurally known sex pheromones for WCR and SCR. Sex pheromones are probably not a reproductive isolating mechanism for these rootworms (Dobson & Teal, 1987); the shared pheromone explains observed field pairings between NCR males and WCR females (Ball, 1957). Post-mating barriers and other factors prevent significant field hybridization between species (Krysan & Guss, 1978).

In flight tunnel studies (Dobson & Teal, 1987), the threshold release rate for male WCR flight toward the pheromone was between 0.44 and 1.18 ng/h (rubber septa were loaded with 0.1 and 0.25 µg/lure at 24 °C). Guss *et al.* (1982) reported that the threshold dose for male response was approximately 10 ng/lure in field trials (release rate was not reported). Guss *et al.* (1984) describe a male dose response to synthetic

8R, 2R that increased steadily with increasing lure-loading rate from 0.25 µg to the maximum of 1000 µg; Guss *et al.* (1982), had earlier reported a similar increasing response to traps baited with 0.001–100 µg/trap.

In the hours after adult emergence, young females crawl up nearby maize plants or fly short distances to nearby plants where they feed, groom or rest motionless (Quiring & Timmins, 1990). This is consistent with the calling posture described by Hammack (1995) who reported that, during calling, the tip of the abdomen may be everted exposing the dorsal and ventral intersegmental membranes between the seventh and eighth abdominal segments. The exposed area is the same as the sex pheromone release site identified by Lew and Ball (1978) as secretory-type epithelial cells (with associated cuticular pore openings) at the tip of the adult female WCR abdomen. Hammack (1995) reported that females in this posture were significantly more likely to be sexually receptive to males than those who were not. Among newly-emerged females, 54% began calling on their emergence day, 96.4% called during the next day and all had called by the third day. Laboratory females called throughout the LD 14:10h photoperiod but were most active during the first half of photophase. However, Hammack (1995) noted considerable calling-pattern variability between individuals; only approximately 80% of females were attractive to mate-seeking males. In field studies using sticky traps baited with virgin females, Bartelt and Chiang (1977) could not identify any calling pattern based on trap capture data.

A current calling posture was not necessary for a male to locate a female; 70% of females that mated during observations were not calling at the time they were approached by mate-seeking males (Hammack, 1995). Although unmated females continued to call for several days in the Hammack (1995) study, an extended period of virginity for field females is unlikely given a normal abundance of males. Several authors report that calling females are rapidly mated (Ball, 1957; Hill, 1975; Lew & Ball, 1979). A high proportion of teneral females [i.e. their elytra are pale-coloured because the 12–24h post-emergence sclerotization process is incomplete (Cates, 1968)] in mating pairs supports the rapid mating hypothesis. Quiring and Timmins (1990) reported that 96.6% of mating females were teneral (97.2% of males were *not* teneral).

Response to pheromone and mating

WCR antennae are sexually dimorphic; the male flagellar segments are longer and bear 25% more sensilla than the female flagellum (Staetz *et al.*, 1976). There is also a sexual dimorphism in expression of Est-a, a possible odourant metabolizing acetylcholinesterase found only in the five most distal segments of male antennae (Newman *et al.*, 1993). The structure and distribution of sensilla on the male antennae suggest sex-specific specializations for pheromone detection (Staetz *et al.*, 1976; Newman *et al.*, 1993).

Field males exhibit bimodal morning and evening peaks of activity in response to pheromone sources (Bartelt & Chiang, 1977; Dobson & Teal, 1986); the greatest laboratory response was in the morning (Dobson & Teal, 1987). Initial male perception of pheromone causes agitation; males also raise their

antennae and move them back-and-forth excitedly (Lew & Ball, 1979). Responding males orient their bodies into the pheromone plume and take flight (Lew & Ball, 1979). Dobson and Teal (1987) characterized a slow hovering flight with the body held in a vertical orientation as a positive response to pheromone. WCR males in laboratory arenas with calling females extended their antennae forward and approached a potential mate from the rear, using antennae to touch her abdomen (Lew & Ball, 1979).

Once in contact, the male may lunge or jump onto the female and grasp her elytra with the first two pairs of legs as the hind legs rest on the substrate. An unreceptive female may discourage a mounted male by turning her abdominal tip downward or by attempting to dislodge him by kicking and walking away (Lew & Ball, 1979). If not immediately rejected, the mounted male will continue physical courtship activity for 10–60 min before copulation (Lew & Ball, 1979). During the pre-copulatory period, he may repeatedly tap or stroke the female head and antennae with his antennae. In the spotted cucumber beetle (SCB) *Diabrotica undecimpunctata howardi* Barber, the stroking behaviour promotes relaxation of the female vaginal duct allowing the male's needle-like aedeagus to access the bursa copulatrix and begin delivering the liquid spermatophore components (Tallamy *et al.*, 2002a). A faster stroking rate in SCB males is associated with a greater likelihood of being accepted as a mate (Tallamy *et al.*, 2002b). Lew and Ball (1979) report that WCR antennal tapping quieted the female allowing the male to insert his aedeagus. Among less-receptive females, the male tapping may become rapid and be followed by aggressive thrusting of the aedeagus into the opening of the female reproductive tract. Once *in copulo*, the male bends his abdominal segments downward and his antennae cease moving and are folded backward over the male elytra. In SCB, posterior folding of the antennae over the elytra is a sign that a spermatophore is being successfully transferred (Tallamy *et al.*, 2000). Periodically during WCR copulation, males stroke the sides of the female with the mesothoracic legs; Lew and Ball (1979) suggest this is also to calm the female.

During copulation, a WCR male resists female attempts to dislodge him by anchoring his metathoracic legs under the tip of the female abdomen. Hairless, planar ovoid patches found only on the basitarsi of the prothoracic and mesothoracic male legs of WCR and NCR may aid males in gripping the female elytra and maintaining a mating posture (Hammack & French, 2007). Periodically males engage in vigorous side-to-side rocking motions, punctuated by less intense forward and backward thrusting. When *in copulo*, the female may walk about and feed or groom; males have no opportunity to feed. WCR mating lasts 3–4h (Ball, 1957; Lew & Ball, 1979; Sherwood & Levine, 1993) whether in the laboratory or field (Lew & Ball, 1979). At its conclusion, the male may remain atop the female in a mate-guarding posture until she disturbs or dislodges him.

Spermatophore

A mating male deposits a large spermatophore in an enlarged muscular pouch posterior to the females's common oviduct called the bursa copulatrix (Lew & Ball, 1980). Spermatophores are produced from proteinaceous secretions

of the male accessory glands that are used to transfer sperm to the female (Gillott, 2003). The WCR spermatophore consists of two parts: a milky, gelatinous portion with a layered structure deposited in the anterior lobe of the bursa during the first hour of mating, and a pale pink spherical portion that is later deposited in the bursa's posterior lobe (Lew & Ball, 1980). Sperm are stored in a dark, sclerotized, sickle-shaped spermathecae that is ducted to the bursa copulatrix [WCR internal anatomy is similar to that of SCB (Mendoza and Peters 1968)]. Within 2.0 h after initiation of mating, 35% of females have some sperm stored in their spermatheca; 100% have some stored sperm by 4.0 h (Lew & Ball, 1980). Some sperm can be found in the slowly degrading spermatophore for up to 3 days after mating; no trace of the spermatophore remains after approximately 5–7 days (Lew & Ball, 1980). Based on production of viable eggs, stored sperm are viable for ≥ 40 days and perhaps ≥ 76 days (Branson & Johnson, 1973; Hill, 1975; Lew & Ball, 1980).

Quiring and Timmins (1990) measured WCR mating frequency and spermatophore mass for ten large (mean \pm SEM: 11.71 ± 0.47 mg) and ten small (6.03 ± 0.20 mg) males. Larger males initiated their first mating at an earlier age (2.0 versus 3.2 days) and mated more frequently (15.8 ± 1.2 versus 9.7 ± 2.1 times) than smaller males. Peak male mating activity during weeks 2 and 3 corresponded with peak female emergence (Quiring & Timmins, 1990). Branson *et al.* (1977) reported that males mated 8.2 times in 41.6 days and one mated 14 times. Hill (1975) reported that males mated with up to four females in a day. Some large and small males in the Quiring and Timmins (1990) study mated twice per day; no males mated three times in a day. Surprisingly, mean spermatophore size was not different between large (0.57 ± 0.02 mg) and small males (0.54 ± 0.03 mg). When males mated twice a day, mean spermatophore size was not different from that when only one was produced during a day (0.50 ± 0.04 mg) (Quiring & Timmins, 1990). Depending on male size, deposition of the spermatophore involves transfer of material equal to 5–9% of the male's mass, a percentage similar to the 7% of male mass transferred to females during SCR mating (Tallamy *et al.*, 2000). Some twice-mating males transferred up to 24% of their initial body mass to their mates (Quiring & Timmins, 1990). Rarely, females with two spermatophores are found (Branson *et al.*, 1977; J.L. Spencer, unpublished data).

In many insects, the mating act and/or substances transferred in semen or the spermatophore reduce female mating receptivity and enhance fecundity (Gillott, 2003). Hammack (1995) reported that calling behaviour was suppressed in mated females; only one of 30 was observed in the calling posture. When matings are terminated before sperm were transferred, Sherwood and Levine (1993) found that females had an increased likelihood of laying eggs compared with unmated females. However, interrupted females laid significantly fewer eggs than females from completed matings or interrupted matings where some sperm were transferred. When females mated for just 1 h, an expected increase in egg development (typically observed at approximately 10–16 days post-mating) quickly waned (Sherwood & Levine, 1993). Females mated for 1 h laid 72.1% of their total egg output at 6–10 days after mating; normally-mated

females oviposited steadily over the first 27 days post-mating (Sherwood & Levine, 1993). In this study, no eggs were laid within 5 days of mating; females were 6–10 days old when oviposition began (Sherwood & Levine, 1993). Other studies report pre-ovipositional periods ranging from 12.2 ± 2.0 days (mean \pm 95% confidence interval) (Hill, 1975), 14.3 days (Branson & Johnson, 1973) to 21 days (Short & Hill, 1972). Given reduced oviposition among incompletely mated females, diminished volume of semen or spermatophore components reduces fertility, whereas just the act of mating can increase oocyte development (Sherwood & Levine, 1993). The fecundity-enhancing effect of mating was observed in NCR \times WCR crosses; although egg production was increased, there was low viability (Krysan & Guss, 1978; Hintz & George, 1979). The same is true for WCR \times MCR crosses (Krysan *et al.*, 1980). Reproductive incompatibility due to *Wolbachia* infection probably contributes to isolation between the *Wolbachia*-infected WCR and the *Wolbachia*-free MCR (Giordano *et al.*, 1997). *Wolbachia*-based interactions between WCR and NCR are less clear. Clark *et al.* (2001) reported NCR to be *Wolbachia* free; however, Roehrdanz and Levine (2007) sampled more extensively and found two *Wolbachia* strains in NCR populations from east of the Mississippi River. These may be incompatible strains; one of the NCR strains is similar to a *Wolbachia* strain found in WCR (Roehrdanz & Levine, 2007).

One mating probably provides a lifetime supply of sperm (Branson & Johnson, 1973; Hill, 1975); it is adequate to maintain an elevated rate of egg development that supports oviposition for 4–5 weeks after mating (Sherwood & Levine, 1993). It is speculated that old WCR females may remate if their sperm supply is exhausted (Branson *et al.*, 1977). By initially pairing females with sterile (irradiated) males and later presenting untreated males, Branson *et al.* (1977) suggested that females will not remate when they are actively ovipositing. When second matings occurred, subsequent eggs were fertilized by the second male's sperm, suggesting last male sperm precedence; however, Branson *et al.* (1977) question the competitiveness of irradiated male sperm. These results also imply that viable sperm are not necessary to stimulate oviposition.

Significant questions about courtship and mating

The mechanics of mating in *Diabrotica* spp. are perhaps best characterized for the SCR (Tallamy *et al.*, 2000, 2002a, b); research has revealed many parallels between SCR and WCR behaviour. Invoking a similar mechanistic behavioural and chemical ecology-based approach, including attention to the fate of cucurbitacins (bitter compounds that are strong phagostimulants for diabrotic beetle of tribe Lupernini) in WCR mating studies, could yield valuable insights into the intersection of WCR reproduction and cucurbitacin pharmacophagy (Tallamy *et al.*, 2005). Studies by Sherwood and Levine (1993) and others provide evidence for male-contributed fecundity-enhancing substances. Responsible for curtailing female mating receptivity and accelerating post-mating egg development, these male-derived proteins modulate key transitions in the WCR lifecycle. Known as sex peptides, these

substances have been extensively studied in a number of insect orders (Gillott, 2003), notably in Diptera where the *Drosophila melanogaster* sex peptide receptor has been identified (Yapici *et al.*, 2007). It may be possible to use molecular tools and approaches to explore control of calling behaviour and regulation of WCR oogenesis.

A thorough understanding of WCR mating behaviour is one prerequisite for designing an effective IRM plan. Current IRM plans for rootworm-resistant transgenic corn hybrids incorporate the assumption that mate-seeking refuge males will out-compete the scarce transgenic field males for opportunities to mate with females emerging from transgenic corn. However, the literature contains little about WCR reproduction in the field. The maximum distance from which field males respond to calling females, or the impact of calling female density on that distance and male mate-finding success are unknown. The effect of high versus low adult density (e.g. conditions typical of refuge versus *Bt* maize fields) on male-female interactions is also not known.

Other significant questions are also related to assumptions about mating and refuge function. How frequently do males mate in the field and how long must the refractory period between matings be to assure that a female can be fully provisioned with sperm and spermatophore-forming proteins during mating? Do males seek mates when they are incapable of depositing a full spermatophore? How would a limited male mating potential affect IRM assumptions for transgenic maize systems? Do females have any role in determining the duration of copulation, or an ability to extend it to prolong transfer of male contributions? In what proportion and at what age do females in the field remate, if ever? If remating is ecologically relevant, what sort of sperm precedence is there among WCR?

Oviposition

Bayar *et al.* (2002) observed a WCR pre-ovipositional period of 5–42 days. Under normal conditions, females passed through a 13-day pre-ovipositional period, eggs were then oviposited for 60 days during the first ovipositional period (Branson & Johnson, 1973; Hill, 1975; Fisher *et al.*, 1991). If oviposition stops, Branson *et al.* (1977) observed that a second nonovipositional period continues until death, unless an uncommon second mating occurred.

Mean lifetime fecundity of once-mated WCR females held under realistic, but close to optimal nutritional conditions, was 440 viable eggs (Boetel & Fuller, 1997). Elliott *et al.* (1990), Ball (1957) and Fisher *et al.* (1991) observed mean fecundities of 441 total eggs, 372 eggs/female (in 1954), 418 eggs/female (in 1955) and 266 viable eggs, respectively. Similar fecundity was found for Hungarian WCR; Bayar *et al.* (2002) observed a mean \pm SD fecundity of 226 ± 133 eggs per female, whereas Toepfer and Kuhlmann (2006) report a mean \pm SD fecundity of 353 ± 237 . If diet is supplemented, very high mean fecundity [i.e. 1023 ± 240 eggs (Branson & Johnson, 1973) and 1087 ± 217 (Hill, 1975)] is possible. Over an 8-week oviposition period, the percentage of eggs that hatch (after diapause termination) declines from approximately 80 to 30% (Fisher *et al.*, 1991).

Historically, WCR oviposition and feeding took place almost exclusively in maize fields (Shaw *et al.*, 1978; Levine & Oloumi-Sadeghi, 1991). Branson and Krysan (1981) concluded that females lay eggs wherever they are feeding. Before rotation resistance, most economic injury to rotated maize roots was attributed to NCR (Steffey *et al.*, 1992) or to WCR oviposition around attractive weeds or volunteer corn in soybean (Shaw *et al.*, 1978). In retrospect, some of that injury was probably due to natural variability in WCR ovipositional site selection (Spencer *et al.*, 2005). European studies of invasive WCR in maize and rotated crops reveal their presence and oviposition outside of maize. However, this occurrence is explainable based on normal patterns of movement and incidental oviposition; rotation resistance is not present (Kiss *et al.*, 2005; Barcic *et al.*, 2007). Knight *et al.* (2005) reached a similar conclusion regarding WCR abundance in soybean from South Ontario, Canada.

Rotation-resistant WCR populations have lost their former ovipositional fidelity to maize fields (Levine *et al.*, 2002). In studies of rotation-resistant WCR in Illinois, Rondon and Gray (2004) found gravid females in maize, soybean, oat (*Avena sativa* L.) stubble, and alfalfa (*Medicago sativa* L.) throughout the growing season; eggs were recovered in all crops. Schroeder *et al.* (2005) also observed rotation-resistant adults and found eggs in maize, soybean, wheat and wheat double-cropped with soybean, although egg densities were highest in corn. Pierce and Gray (2006b) determined that 60% of Illinois WCR eggs are laid in the top 10 cm of soil when 20 cm samples are taken from either maize or soybean fields. An earlier Iowa study by Gray and Tollefson (1988) found 68% of WCR eggs in the bottom 10 cm when 20 cm samples are taken from maize. Pierce and Gray (2006b) concluded that the rotation-resistant WCR oviposits in maize, soybean, oat stubble and alfalfa. Thus, the lack of a distinct ovipositional preference among rotation-resistant WCR probably contributes to widespread oviposition in all crops.

The simple requirements that stimulate oviposition may have enabled a broadened ovipositional host range. Kirk *et al.* (1968), Gustin (1979), and Kirk (1979) report that almost any moist particulate substrate is an acceptable WCR ovipositional site. Odours from homogenates of WCR ovaries or male abdomens, excised maize roots, bacterial cultures and pure CO₂ all influenced choice of ovipositional site (Lance, 1992). Because the homogenates were deactivated by sorbate, a bacteriostatic agent, most treatments were probably active due to enhanced levels of CO₂. In the field, drought cracks, open earthworm burrows and other crevices (i.e. cracks around cornstalks) provide passageways for WCR to locate suitable damp locations (Kirk, 1979, 1981a, b). WCR do not dig burrows (Kirk, 1979).

Periodicity

Conditions that influence expression of periodicity

WCR flight activity is limited by environmental extremes of temperature, windspeed, solar radiation (including day/night cycles) and precipitation. Conditions that are too cold

(< 15 °C), too warm (> 32 °C), too windy (> 2.0 m/s), rainy, or dark will prevent WCR adults from flying within or between fields (Isard *et al.*, 1999). Adult emergence or intrafield flight activities, for which expression is probably gated by a circadian oscillator, may be delayed by unfavourable conditions. Dobson and Teal (1986) described how evening and nighttime temperatures near thresholds for beetle activity (15 °C) suppressed normal expression of WCR and NCR responses to pheromone-baited traps. An expected period of NCR activity was shifted to the next morning where it overlapped with the peak of WCR activity (Dobson & Teal, 1986). Ball (1969) found that adult WCR insecticide (diazinon) sensitivity varied with photoperiod; field-collected WCR were most vulnerable to insecticide from midday to late evening.

Locomotor activity

Van Woerkom *et al.* (1980) measured adult WCR locomotor activity patterns in a laboratory actograph. At constant temperature, WCR adults were most active between 17.00–08.00 h, and had reduced activity between 09.00–17.00 h; a pattern similar to that recorded by Witkowski *et al.* (1975). Van Woerkom *et al.* (1980) noted that peak male activity occurred at a slightly cooler temperature (25–27 °C) than female activity (27–29 °C); overall, males were also more active than females.

Adult emergence

Quiring and Timmins (1990) present nearly overlapping patterns of daily male and female adult emergence in Ontario maize fields. Peak emergence for both sexes occurred at approximately 08.00 h with a secondary peak at approximately 20.00 h. The emergence minima for both sexes occurred between 12.00 and 16.00 h. An increase in the observed number of mating pairs that was coincident with peaks of adult female WCR emergence provides circumstantial evidence for the rapid mating of emerged females.

Flight, interfield movement and abundance in crops

Local adult WCR field abundance and sex ratios reflect the balance between inter- and intrafield movement by WCR adults. In areas where rotation-resistant WCR are present, frequent, season-long interfield movement between maize fields and other crops leads to widely distributed WCR populations. High WCR abundance in first-year and continuous maize, along with high populations and a female biased sex ratio outside of maize fields are characteristic of areas where rotation resistance is present (O'Neal *et al.*, 1999; Levine *et al.*, 2002; Schroeder *et al.*, 2005; Spencer *et al.*, 2005). Factors that influence field-to-field movement affect daily patterns of abundance. In regions without rotation-resistance, abundant WCR adults from continuous maize fields eventually disperse into rotated maize creating female-biased populations of WCR adults (Godfrey & Turpin, 1983).

Interfield WCR flights typically occur during morning and evening (Witkowski *et al.*, 1975; Coats *et al.*, 1986; Grant & Seevers, 1990; Naranjo, 1990b; Isard *et al.*, 2000). Witkowski *et al.* (1975) used beetle counts on sticky traps to infer that WCR flight activity in Iowa maize fields was bimodal, with peaks of greatest flight occurring during the hours just before sunset and the hours just after sunrise. Temperatures between 22.2–27.0 °C were characteristic of the periods with greatest flight activity. Naranjo (1990b) used a vertical flight mill design (Wales *et al.*, 1985) to measure diurnal trivial flight activity patterns among tethered males and females in the laboratory. Although the activity peaks and troughs in this well-replicated study are not distinctly resolved, bimodal morning and evening peaks of flight activity remain evident. Where populations are high, interfield WCR flights can involve substantial numbers of individuals. Extrapolation of captures from eight malaise traps positioned at the borders of a 1.64-ha soybean field in east central Illinois suggests that 0.25 million rotation-resistant WCR crossed the borders of the field on 1 day (Isard *et al.*, 2000).

When rotation-resistant WCR are present in an area, there will be a strong daily abundance periodicity in soybean fields (and other rotated crops) associated with periods of peak interfield flight activity at field margins (Spencer *et al.*, 1999b; Isard *et al.*, 2000). Peak populations of WCR in soybean fields are found in the morning and evening, with significantly lower WCR abundance during midday (Spencer *et al.*, 1999b). The direction of interfield movement was inferred by the detection of ingested maize and soybean tissues in WCR gut contents. The proportion of WCR adults collected in soybean fields containing ingested maize and soybean tissues (indicative of recent movement into soybeans after feeding in a maize field) rises and falls as WCR move in and out of soybean fields during the course of a day (Spencer *et al.*, 1999b, 2005). Return flights from soybean to maize may be aided by the height difference between the crops; in a windtunnel study, flying WCR tended to land on taller plants in their flight path without preference for plant species (Spencer *et al.*, 1999a).

Flight periodicity also affects the dispersal of recently mated females ascending from their natal maize fields. Netted from 10-m tall scaffolding towers, dispersing WCR females are smaller, younger, and carry less-developed oocytes than females collected within crop canopies or flying above the soybean canopy (Spencer *et al.*, 2005). Like females nearer to the ground, they fly in the early to mid-morning or near dusk (Isard *et al.*, 2004). Although high winds or low temperatures may limit flight, under conditions otherwise conducive to flight, flight activity was not strictly dependent on the value of any particular environmental factor. Isard *et al.* (2004) observed that the flying WCR flux at 10 m rose and fell as atmospheric conditions (measured with a nearby weather station) just above the plant canopy became more or less favourable for WCR ascent. In the lower 10 m of the atmosphere, it is likely that the flying population ascended from nearby.

Ovipositional periodicity

The generally hidden location [within soil drought cracks, earthworm burrows and other soil openings (Kirk, 1979, 1981a, b)] of ovipositional sites may explain why there are

no published field accounts of diurnal periodicity in WCR oviposition. Kirk (1981a) suggests that oviposition is a nocturnal activity and that females using earthworm burrows as ovipositional sites enter them at night. Ball (1971) published results from a laboratory study showing that peak WCR oviposition occurred between 08.00–1200 h. Ball (1971) hypothesized that the combination of general activity patterns (suggested to peak between 16.00–20.00 h) with soil moisture, shown by Cates (1968) to influence the number of eggs laid by females, may explain ovipositional timing.

Significant questions about periodicity

Despite the relevance of daily WCR activity to oviposition and feeding within and between maize fields, other crops and noncrop fields, there are no detailed time budgets for WCR behaviour in the field. In the rotation resistance area, how long do females and males stay in a soybean or other nonhost field before they must return to maize? Does poor diet or imminent oviposition change expression of periodic behaviour? Is the daily periodicity of oviposition in maize different from that in soybean or other fields? The expansion of rotation-resistant WCR populations in the U.S.A. has increased the abundance of egg-laying WCR beetles in fields of rotated crops. There is interest in adult WCR control in soybean (and other rotated crops) to reduce oviposition and potentially manage the risk of larval injury when that field is rotated to maize during the next year. However, the periodicity of WCR interfield movement between maize and rotated crops makes this unlikely to succeed: beetles killed on one day are rapidly replaced by new immigrants. Are there occasions when conditions would be favourable to target adult control against WCR in non-maize settings? Is it possible to forecast conditions likely to promote long-distance or local dispersal and take action to protect or target particular fields?

Nutritional ecology

Many aspects of the nutritional ecology of WCR, including density-dependent survival were reviewed by Moeser and Hibbard (2005). This included the range of alternative host plants used by WCR in the U.S.A. and Europe (Clark & Hibbard, 2004; Moeser & Vidal, 2004a; Oyediran *et al.*, 2004), which was reviewed to assess potential adaptations to transgenic *Bt* maize in the U.S.A. (Wilson & Hibbard, 2004; Chege *et al.*, 2005) and to assess potential host shifts in European agroecosystems. They covered the latest work on plant compounds responsible for food conversion efficiency, including the C/N ratio and the phytosterol content (Moeser & Vidal, 2004b) and the role of hydroxamic acids (Xie *et al.*, 1990, 1992a, b; Assabgui *et al.*, 1993, 1995a, b; Arnason *et al.*, 1997) in WCR larval performance. Because adult and larval diet and patterns of feeding affect adult and larval movement, appropriate reference to WCR nutritional ecology is included in some sections dealing with movement (see below).

Transgenic maize with resistance to corn rootworms specifically targets neonate larvae via expression of insecticidal proteins from *Bt*; multiple resistant lines have been devel-

oped (Moellenbeck *et al.*, 2001; Vaughn *et al.*, 2005). Larval control has also been achieved via RNA interference in transgenic maize expressing double-stranded WCR RNAs (Baum *et al.*, 2007). The *Cry3Bb1* protein in MON863 rootworm transgenic maize (Vaughn *et al.*, 2005) and the *Cry34Ab1*/*Cry35Ab1* proteins in Event 59122 (Storer *et al.*, 2006) are both expressed in roots and throughout the plant (US-EPA, 2003, 2005). The possible impact of chronic *Cry3Bb1* protein exposure due to adult WCR feeding on above ground plant parts was investigated by Al-Deeb and Wilde (2005). Nowatzki *et al.* (2006) examined effects of *Cry3Bb1* exposure, but used cellulose disks with different *Cry3Bb1* protein concentrations treated with a feeding attractant. In both studies, the authors demonstrated that the *Cry3Bb1* protein had no adverse effects on adult rootworm longevity and other performance measures. In the study by Al-Deeb and Wilde (2005), this was true even for adults that developed on MON863 maize as larvae. Oyediran *et al.* (2005, 2007) investigated interactions of alternate hosts with transgenic maize expressing the *Cry3Bb1* protein. Although significantly more WCR adults emerged from *Bt* corn + grassy weeds than from *Bt* corn alone or weeds alone in the greenhouse, the effect was not as dramatic in the field.

Significant questions about nutritional ecology

What influence will the varied European food sources and more diverse agricultural landscape have on the spread and persistence of invasive WCR adults? Are novel host associations to be expected in Europe for adults and larvae? Are there larval nutritional requirements that could that be exploited in breeding of resistant maize hybrids? Metcalf and Metcalf (1992) provide a detailed study of semiochemicals that influence adult host finding and acceptance, extending these studies to include cues that trigger or impede alighting and/or host acceptance may yield new management and monitoring tools. Rootworm-resistant transgenic maize hybrids expose feeding WCR adults to the same *Bt* proteins that kill WCR neonates. Are there sublethal effects of chronic adult field exposure to transgenic pollen and silk for larvae that developed on refuge or *Bt* maize? As more transgenic hybrids are commercialized, adults will be exposed to multiple different *Cry* proteins. Will adult feeding on tissue from multiple transgenic hybrids alter fecundity of females emerging from refuge versus transgenic maize fields? Is mate-seeking male movement, the quality of spermatophores, or the post-mating refractory period affected by adult diets that include transgenic hybrids?

Movement

Larval movement

After egg hatch, physical and chemical factors interact to affect first-instar larval movement and subsequent establishment on maize roots. Because the first instar is small and soft-bodied, movement throughout the soil is probably accomplished by following interconnected air-filled pores in the soil with little actual burrowing (Gustin & Schumacker, 1989).

Larval movement is limited by increasing soil bulk density (Strnad & Bergman, 1987a; Ellsbury *et al.*, 1994) and pore sizes smaller than larval head capsule width (Gustin & Schumacker, 1989). Pore size distribution is directly related to the texture, structure, and soil bulk density. First instars move farther in fine textured soils than coarse soils (MacDonald & Ellis, 1990). The interaction of soil texture and moisture affects movement. Within a soil texture, very wet or dry soil conditions can restrict movement of first instars (MacDonald & Ellis, 1990). Very wet conditions can reduce available air-filled pores as many spaces become saturated with water. Establishment success on maize plants is independent of egg density (Hibbard *et al.*, 2004), but might be affected by the number of infestation points around a plant (Wilson *et al.*, 2006). In artificial infestations with varying infestation depths, infestation rates, and distances from a maize row, Chaddha (1990) documented that there was no significant difference in adult emergence between infestation depths of 7.5 or 15 cm. However, as distances from the maize row increased, plant damage decreased. Adult emergence was also affected by the distance between the maize row and where eggs were infested, but to a greater extent at the lower infestation rate, perhaps because of density-dependent mortality at the higher infestation rate closer to the maize.

Larval migration is not complete when the neonate reaches a host plant. Strnad and Bergman (1987b) demonstrated that as larvae grow, they are redistributed, moving to younger root whorls that emerge from the stalk. Hibbard *et al.* (2003) documented that WCR larvae can move at least three plants down a maize row and across a 46-cm row after initial establishment. Although egg density appeared not to be an important factor in percent larval establishment (Hibbard *et al.*, 2004), it was an important factor in plant damage and, secondarily, subsequent larval movement. In general, damage was highest in the plots infested with the most viable eggs and decreased with increasing distance from the infested plant (the infested plant was surrounded by plants without rootworm infestation). Post-establishment movement generally occurred about the time that significant damage began to appear, rather than at the time of establishment (Hibbard *et al.*, 2004). This implies that plant-to-plant movement was motivated by a search for food and was density-dependent only because damage was density-dependent. If crowding rather than a lack of food caused larval movement, it would be expected to have occurred earlier in Hibbard *et al.* (2004). Larval movement from egg hatch to adult emergence is reported to be as great as 100 cm (Suttle *et al.*, 1967; Short & Luedtke, 1970). Although Branson (1986) questioned these results for procedural reasons, Hibbard *et al.* (2004) document movement as far as 61 cm; movement up to 100 cm from egg hatch to adult emergence is conceivable. Compaction of interrow soil from wheel traffic can prevent larval movement into maize from soybean areas planted to maize the previous year in a strip intercropping system (Ellsbury *et al.*, 1999).

Significant questions about larval movement

The value of information about WCR larval movement has increased with commercialization of rootworm-resistant

transgenic maize hybrids. Location of host roots by neonates moving through the soil is essential if WCR populations are to persist. Despite the pivotal nature of this initial host-finding phase, many details about larval movement and factors that limit it are yet to be understood. The true extent of burrowing by neonates, if any, versus movement through existing soil pores and air-filled channels is not known. Under laboratory conditions, food is often limited. When this occurs, moving larvae can travel on top of moist soil. If this behaviour occurs in agroecosystems, starving larvae may move greater distances than expected. Larval movement has been hypothesized to play a role in the potential for development of resistance in a seed mix refuge of *Bt* maize. Is the frequency of this movement great enough to constitute a real resistance threat? What type of selection pressure is placed on larvae with partial exposure to *Bt* maize?

Why do most viable eggs not produce larvae that become established on a host plant? The cause of mortality for most viable eggs in the field is not clear. Egg establishment success in the field is only approximately 5–10% (Hibbard *et al.*, 2004). Establishment success for artificially infested neonates in greenhouse conditions can be 50% (Clark & Hibbard, 2004; Oyediran *et al.*, 2004; Wilson & Hibbard, 2004); establishment success for eggs under greenhouse conditions is approximately 20% (Weiss *et al.*, 1985). Significant mortality is occurring between egg hatch and movement to a root and larval establishment; what factors are responsible for this mortality?

Adult movement: intrafield activity

Many methods are used to study WCR movement within and between maize fields, including mass marking techniques (Lance & Elliott, 1990; Naranjo, 1990a; Oloumi-Sadeghi & Levine, 1990; Spencer *et al.*, 1999a; Toepfer *et al.*, 2006). Recently, Rubidium labeling of maize plants (Nowatzki *et al.*, 2003) and detection of ingested transgenic maize tissue (Spencer *et al.*, 2003) were developed as movement monitoring techniques. Unlike traditional marking methods, WCR acquire the detectable transgenic proteins or Rubidium-labeled tissues during normal normal feeding on maize plants in the field. The self-marking feature of both techniques is attractive when high WCR populations prevent hand-marking of an informative proportion of the population. However, since the number of marked insects is unknown, the techniques aren't useful to estimate population size from marked insect data.

Detection of ingested transgenic maize tissue as described in Spencer *et al.* (2003) is in use to study intrafield movement of WCR between transgenic maize fields and adjacent strips of nontransgenic maize. Average intrafield movement rates for males range between 6–17 m/day (J.L. Spencer, unpublished field data), female intrafield movement rates were not different from those of males. Tethered flight mills were used to measure short and sustained WCR flight capabilities (Coats *et al.*, 1986; Naranjo, 1990a).

In their first hours, most calling, teneral females move only short distances from their emergence sites before their they are rapidly intercepted by mate-seeking males (Ball,

1957; Hill, 1975; Lew & Ball, 1979; Quiring & Timmins, 1990). After mating, female WCR remain in their natal maize field to feed for a few days to a week before many ascend and disperse to a different maize field during morning or evening periods of WCR flight activity (Isard *et al.*, 2004). Based on samples of flying WCR collected from atop 10 m tall scaffolding towers, dispersing populations of WCR are 85% female, 84% of these females contained a spermatophore, 43% of those were large spermatophores (Isard *et al.*, 2004). Because a spermatophore is slowly absorbed during 5–7 days after mating (Lew & Ball, 1980), the presence of one, or the collapsing remnants of one, is an indication that a female was recently mated. Low body mass and poorly developed oocytes in these females also indicate they are young and recently-mated (Isard *et al.*, 2004). Though the flight duration or destination of dispersing WCR in the Isard *et al.* (2004) study could not be measured, the youthful, pre-ovipositional characteristics of the flying individuals combined with the morning or late evening occurrence of flight suggest these WCR were initiating sustained migratory flight as described by Coats *et al.* (1986).

Although males move extensively in maize to locate mates, their low representation among dispersing WCR at 10-m elevation (Isard *et al.*, 2004) suggests that field males are less likely than females to initiate sustained flight. However, in laboratory flight mill studies, sustained flight parameters like number and duration of flights were similar between the sexes (Naranjo, 1990b; Stebbing *et al.*, 2005). The proportion of individuals that make sustained flights on flight mills range from < 24% (Naranjo, 1990b), 15% (Coats *et al.* (1986) to approximately 1.5% (Stebbing *et al.*, 2005).

Adult interfield movement

The arrival of female-biased WCR populations that have dispersed from their natal fields establishes female-biased sex ratios for WCR populations in rotated maize (in areas without rotation-resistant WCR) and non-maize rotated soybean and other crops (in areas with rotation-resistant WCR). Data from Hill and Mayo (1974) and Godfrey and Turpin (1983) illustrate how female-biased WCR populations begin to colonize first-year maize approximately 1 week after emergence and dispersal from continuous maize. Few WCR were ever found outside of maize; in fact, the NCR was considered the more mobile rootworm (Hill & Mayo, 1980).

Surprisingly similar patterns of WCR arrival, relative abundance, and sex ratios are now characteristic of rotation-resistant WCR colonization of soybean and other crops from their natal first-year maize fields (O'Neal *et al.*, 1999; Levine *et al.*, 2002; Rondon & Gray, 2003; Pierce & Gray, 2007). The unsuitability of soybean as an adult WCR host (Mabry & Spencer, 2003) has introduced the necessity of frequent interfield movement from soybean to maize to remedy the nutritional stress associated with soybean herbivory (Mabry *et al.*, 2004).

Beyond the initial female bias in the sex ratio of the WCR populations that settle in new maize fields, WCR females are distributed higher in the maize canopy than males (Witkowski *et al.*, 1975; Van Woerkom *et al.*, 1983), which may predis-

pose them to interfield trival flights. Females are more abundant than males among WCR flying between fields at ground level [75% of WCR collected between maize fields (Lance *et al.*, 1989); 67% of WCR collected between maize and soybean fields (Isard *et al.*, 2000)]. The tendency of females to leave continuous maize for rotated maize is evident in comparisons of percent female measured in continuous versus rotated maize (32.9% versus 74.7% (Levine & Gray, 1994); 50.4% versus 65.3% (Godfrey & Turpin, 1983); Hill & Mayo (1974) present similar patterns. Where rotation resistance is present, evidence of the same tendency for females to leave their natal fields is found in the percentage female WCR in rotated maize (42.5%) versus rotated soybean (73.4%) (O'Neal *et al.*, 2000).

An increased propensity for interfield movement and a relaxed ovipositional fidelity to maize fields distinguish rotation-resistant WCR populations from rotation-susceptible ones. Broad adoption of annual crop rotation in the eastern U.S. Corn Belt is believed to have selected for existing WCR phenotypes with reduced ovipositional fidelity to maize fields (Levine *et al.*, 2002; Spencer *et al.*, 2005). Over a 20-year period, the reproductive benefits accruing to females leaving maize fields to deposit at least some eggs in soybean and other rotated crops, selected for a WCR population with little ovipositional fidelity to maize fields. In a landscape where 95–98% of soybean is in an annual rotation with maize (Onstad *et al.*, 1999), WCR females that deposit eggs outside of maize fields will produce more surviving offspring than females with perfect fidelity to maize fields. In the case of rotation resistance, selection did not broaden ovipositional host specificity to include new plants: rather, a strict ovipositional fidelity to maize fields was relaxed.

Once dispersing females settle in a new maize field, they resume feeding and continue to develop their eggs. Over most of the U.S. Corn Belt, these females will move randomly (Naranjo, 1994) within the maize field when they forage. As the maize matures, they may make occasional interfield flights into phenologically less-mature maize fields (Naranjo, 1994) where feeding resumes. Initiation of interfield flight is stimulated by detection of volatiles from pollinating maize and developing maize silks, unfavourable conditions in the current field (i.e. advancing crop maturity) (Hill & Mayo, 1974; Witkowski & Owens, 1979; Prystupa *et al.*, 1988; Naranjo, 1994; Hammack, 1996; Darnell *et al.*, 2000; Moeser & Vidal, 2005), or opportunities to exploit weedy sources of pollen late in the growing season (Moeser & Vidal, 2005; Campbell & Meinke, 2006). Pollen analyses in Europe revealed that later in the growing season, flowering weeds outside the field became more attractive. Males exploited a wider range of alternative pollen resources, but used each pollen source less intensively than the WCR females (Moeser & Vidal, 2005). This is a reflection of the greater mobility of males versus females, whereas the females use the nitrogen-rich pollen for their egg development. Interfield movement can be substantial; in a mark–release–recapture study set in Hungary, Toepfer *et al.* (2006) found that 3.8–1.5% of released WCR moved 300 m to small maize fields. Spencer *et al.* (2003) measured interfield movement rates of 4.6–9.1 m/day for WCR moving into soybean from an

adjacent maize field. The presence of volunteer maize plants in soybean fields or other rotated crops is highly attractive: many WCR will orient to these plants, sometimes resulting in significant oviposition in their vicinity (Shaw *et al.*, 1978).

Shortly after the WCR are first detected in continuous or rotated maize fields, WCR activity can be observed in rotated maize or nonmaize rotated crop fields as the populations steadily rise (Godfrey & Turpin, 1983; Levine *et al.*, 2002; Pierce & Gray, 2007). Oviposition in soybean is a consequence of rotation-resistant WCR movement into soybean fields. Pierce and Gray (2006b) observed nearly identical patterns of seasonal egg accumulation in rotated maize and soybean fields, indicating that rotation-resistant WCR began steadily depositing eggs soon after they colonized soybean fields. Given the historical influence of maize phenology on WCR movement (Naranjo, 1991), an abundance of rotation-susceptible WCR in a nonhost field near a flowering maize field would be unexpected.

Pierce and Gray (2006b) observed 50% cumulative oviposition by late July or early August. In continuous maize, Hein and Tollefson (1985) observed 50% cumulative oviposition by mid-to-late August; the difference in dates of 50% cumulative oviposition can be partially attributed to commencing their soil sampling 1 month later than Pierce and Gray (2006b). Although rotation-resistant WCR populations do not often display any consistent pattern of ovipositional preference for maize, soybean or other crops, there is a consistent threat of injury for maize planted after any of several different rotated crops (Rondon & Gray, 2004; Schroeder *et al.*, 2005; Pierce & Gray, 2006b). Enrichment of the free air concentration of CO₂ by approximately 48% to predicted 2050 levels in soybean plots increased WCR oviposition compared with adjacent soybean growing under ambient conditions (Schroeder *et al.*, 2006).

Crop phenology has been hypothesized as a factor contributing to the evolution of rotation resistance (O'Neal *et al.*, 2002, 2004). Trends toward earlier maize planting in the U.S. Midwest create conditions where maize is harvestable when soybean fields are sometimes still green. Under these conditions, the difference in phenology can trigger late-season WCR movement from a senescing field into the surrounding landscape to feed on less-mature host plants (i.e. green tissue) or alternative pollen sources. O'Neal *et al.* (2002, 2004) argue that a response to phenology (WCR movement out of maturing maize and into nearby green-leafed soybean fields) is adequate to explain rotation resistance with no need to invoke widespread crop rotation as the selecting force. They conclude that advancing maize phenology drives rotation-resistant WCR from maize into soybean fields. Although WCR abundance in soybean increases over the growing season (O'Neal *et al.*, 1999), a phenology-based mechanism for rotation resistance does not account for the large numbers of WCR adults found in soybean while maize is still an attractive host (Rondon & Gray, 2003). Pierce and Gray (2006a) tested whether phenology differences alone could bring ovipositing WCR into soybean fields in an area without a rotation-resistant WCR population. They planted maize on dates that were one month apart to create extreme differences in maize and soybean phenology in Champaign County and in an area 220 km to the

northwest without rotation-resistant WCR (Warren County, Illinois). The number of eggs laid by WCR in Champaign County, Illinois soybean plots was uniformly high; twice as many eggs were laid in the late maize (planted 15 May) than in early maize (planted 15 April). In Warren County, no WCR eggs were recovered from soybean field soil, and there were no differences in egg recovery from maize fields planted on different dates. Although differences in maize phenology can influence rotation-resistant WCR oviposition, not even an extreme phenological difference was sufficient to stimulate oviposition in soybean by nonrotation-resistant WCR.

During rotation-resistant WCR activity in soybean fields, 55% feed on soybean tissue (Levine *et al.*, 2002). Although readily eaten, soybean tissue does not support WCR egg development (Mabry & Spencer, 2003). However, soybean herbivory is not unique to rotation-resistant beetles; a similar proportion of the much lower WCR population from soybean fields in rotation-susceptible regions also feed on soybean tissue (Levine *et al.*, 2002). In the laboratory, most field-collected WCR that eat only soybean tissue live < 1 week. However, WCR adults eating diets that alternate between maize and soybean tissue, are as vigorous as WCR maintained on a continual diet of maize plant tissues and are just as fecund (Mabry & Spencer, 2003). This corresponds with other laboratory studies showing that feeding a single food source usually resulted in decreased longevity (Siegfried & Mullin, 1990; Mullin *et al.*, 1991). Studies of WCR under natural conditions revealed that dietary mixing of various food sources occurs in the field. (Moeser & Vidal, 2005; Campbell & Meinke, 2006).

Soybean herbivory also affects movement and oviposition. Mabry *et al.* (2004) found that when WCR were switched from maize to soybean diets or vice versa, soybean-feeding WCR were significantly more active than WCR feeding on maize tissues. In a behavioural assay, WCR also laid significantly more eggs on days when soybean tissue was their only available diet, suggesting that soybean herbivory affects likelihood of oviposition (Mabry *et al.*, 2004). Greater activity and oviposition after exposure to soybean tissue may provide the proximate mechanism behind the interfield movement of rotation-resistant WCR from soybean fields to adjacent maize fields (Mabry *et al.*, 2004; Spencer *et al.*, 2005). Knolhoff *et al.* (2006) looked for evidence of movement differences between WCR populations. They found that females from rotation-resistant populations were faster to escape an arena than females from rotation-susceptible populations; a higher baseline activity level in rotation-resistant populations may promote a greater likelihood of WCR departure from maize fields. A diagnostic behavioural bioassay for rotation resistance would have great value; to date, attempts to identify genetic variability linked to rotation resistance have not been successful (Miller *et al.*, 2006, 2007).

Imminent oviposition cannot be the only force leading to interfield movement by rotation-resistant WCR. Gravid females (or those with resources that would eventually permit some egg maturation) account for only 20% of females that fly into soybean from maize fields (Mabry & Spencer, 2003). The season-long presence of many females without mature eggs in soybean fields suggests that oviposition is not the

reason most females leave maize for soybean. Also, because soybean is such a poor WCR food (Mabry & Spencer, 2003), the 80% of females that enter soybean fields without sufficient reserves to complete egg development must return to a maize field to feed before they can oviposit (Mabry *et al.*, 2004). The implication of this nutritional constraint is that WCR movement into soybean fields must be followed by a later return to a maize field (or some food source), or else they will die. Season-long collection of WCR adults and recovery of eggs from fields of dried wheat stubble, illustrate that rotation-resistant WCR will enter extremely inhospitable environments when abundant resources are present in adjacent maize fields (Schroeder *et al.*, 2005).

Adult long distance movement

During the post-mating, pre-ovipositional dispersal period, favourable atmospheric conditions and light winds promote WCR ascent and flight from maize fields (Witkowski *et al.*, 1975; Van Woerkom *et al.*, 1983). The likelihood of these flights peaks during early-late morning and early evening (Isard *et al.*, 1999, 2000). During the passage of summertime storms, some airborne WCR adults may be drawn into storms and carried tens of kilometers before being washed out in rain (Grant & Seevers, 1989). When WCR-bearing storms pass over Lake Michigan, evidence of storm transport can be found in the piles of WCR beetles that wash-up along the waterline (Grant & Seevers, 1989). Storm transport probably contributes to long-distance WCR dispersal across the Corn Belt. Onstad *et al.* (2001) used storm data to model WCR dispersal and concluded that the distribution of rotation-resistant WCR could be accounted for by storm movement and accumulated displacement due to dispersal and trivial movement. A refinement of the original model added consideration of vegetational diversity (presence of nonrotated, nonhost vegetation works against establishment of rotation-resistant WCR by acting as an ovipositional sink) (Onstad *et al.*, 2003).

Without storm transport, sustained flight during preovipositional dispersal may move some WCR long distances. Using WCR tethered in automated flight mills, Coats *et al.* (1986) and Naranjo (1990b) found that the longest sustained flights were 150 min and 240 min, respectively). Converted to distance travelled, the sustained fliers in the Coats *et al.* (1986) study could have travelled up to 24 km in a single flight or 39.4 km in a series of flights during 1 day. Such a capacity for sustained flight in the field would allow individuals to cover great distances in a few days. Although extremely long duration flights were uncommon, the capability for sustained flight illustrates the capacity for WCR to rapidly spread. The WCR range expansion in the U.S.A. during the mid-late 20th Century (Metcalfe, 1983) testifies to WCR mobility.

In both flight mill studies, sustained flight was a phenomenon of younger, mated females; and its likelihood declined as females aged; no sustained female flights occurred after age 6 and 9 days in the Naranjo (1990b) and Coats *et al.* (1986) studies, respectively. Coats *et al.* (1987) later investigated the physiology of these sustained or 'migratory' flights; juvenile hormone levels were found to be important modulators of WCR flight.

Significant questions about adult movement

Laboratory flight mill studies suggest that less than 25% of WCR engage in sustained flight, yet large shifts in sex ratios follow WCR dispersal from their natal fields. What fraction of females leaving their natal field engage in sustained flight, and how far do they move? Do rotation-resistant WCR populations have any greater tendency for post-mating sustained flight? How far do WCR females travel on dispersal flights? What is the proximate mechanism behind departure from maize fields by rotation-resistant WCR? Are movement rates affected by crop type or the diversity of local plantings? How high do WCR fly during dispersal flights? Are European WCR populations likely to be moved significantly by storm-driven dispersal? How might patterns of long and short distance adult movement contribute to the spread or moderation of resistance to transgenic maize or other management practices?

Chemical and physical cues affecting WCR behaviour

Larvae

WCR larvae express a complicated set of behaviours for host location and acceptance. WCR larvae orient toward carbon dioxide (Strnad *et al.*, 1986; Hibbard & Bjostad, 1988; Bernklau & Bjostad, 1998a, b), which is released by respiring roots of maize and other species (Massimino *et al.*, 1980). WCR larvae were attracted to a range of CO₂ concentrations from 2 to 100 mmol/mol, and can detect gradients as low as 12% (Bernklau & Bjostad, 1998a). In soil bioassay choice tests, significantly more neonate WCR larvae were attracted to a synthetic CO₂ with a higher concentration of CO₂ (11.2 mmol/mol) than to growing maize with a CO₂ concentration of 1.36 mmol/mol (Bernklau *et al.*, 2004). Placement of CO₂-generating materials into soil allowed Bernklau *et al.* (2004) to disrupt the host-location ability of neonate WCR larvae in the laboratory and field, thereby preventing larvae from locating the growing roots of maize.

Although Bernklau and Bjostad (1998b) indicated that CO₂ was the only volatile chemical used by neonate WCR larvae in host location, previous reports used second instar larvae in bioassays to isolate and identify maize semiochemicals in addition to CO₂ (Hibbard & Bjostad, 1990). This work indicated that 6-methoxy-2-benzoxazolinone (MBOA) (Bjostad & Hibbard, 1992) and long-chain free fatty acids (Hibbard *et al.*, 1994) were involved in orientation of second instar WCR larvae. Interestingly, as discussed below, these same long-chain free fatty acids have been documented to be an essential component of a feeding stimulant blend for neonate WCR larvae isolated and identified from germinating maize seedlings (Bernklau & Bjostad, 2008). Although, MBOA showed some promise in increasing the efficacy of insecticides in the laboratory, it did not consistently increase the field efficacy of insecticides (Hibbard *et al.*, 1995).

The liquid extracted from germinating maize contains compounds that stimulate feeding by neonate WCR larvae. In feeding bioassays, neonates fed vigorously on paper disks

treated with liquid pressed from maize roots, or with a solvent extract of maize roots, but not on disks treated with distilled water (Bernklau, 2003; Bernklau & Bjostad, 2005). In subsequent studies, the efficacy of thiamethoxam insecticide was significantly increased when feeding stimulants (maize extract) were added (Bernklau & Bjostad, 2005). The amount of insecticide required for 50% mortality of WCR larvae (within 30 min) was 1 ppm when maize extract was added. With the water control, the highest mortality rate obtained (within 30 min) was only 40%; this required ten-fold the insecticide concentration tested with feeding stimulants. Larval mortality after 24 h was significantly higher for maize extract-treated disks with 0.01, 0.1, 1 or 10 p.p.m. thiamethoxam than for the same concentrations of insecticide with just water. The insecticide required for 50% larval mortality after 24 h was reduced from 1 p.p.m. without maize extract, to 0.01 p.p.m. when maize extract was present. This experiment demonstrated a 100-fold increase in the efficacy of thiamethoxam with the addition of larval feeding stimulants (Bernklau, 2003; Bernklau & Bjostad, 2005). Further unpublished work indicated that the efficacy of thiamethoxam on clay granules in soil could be increased with the addition of feeding stimulants (E.J. Bernklau, personal communication). Tefluthrin and other insecticides commonly used on clay granules were not evaluated. The compounds responsible for the feeding stimulant effect have been identified as a combination of simple sugars (30:4:4 mg/mL glucose:fructose:sucrose in the maize root), plus at least one of the free fatty acids in germinating maize roots (2:5 mg/mL oleic acid:linoleic acid in the maize root) (Bernklau & Bjostad, 2008).

Strnad and Dunn (1990) documented that the behaviour of neonate WCR larvae was dramatically different after removal from a 5-min exposure to the roots of hosts than after a 5-min exposure to the roots of nonhosts. Neonates exposed to host roots exhibited a localized searching behaviour characterized by relatively slow travel with increased turning and path crossings. Neonate WCR larvae exposed to nonhosts exhibited a ranging behaviour with increased rates of travel, fewer turns and path crossings that covered a greater overall area. The Strnad and Dunn (1990) host-recognition factors are unknown, though they have been documented in organic extracts of maize seedlings (E.J. Bernklau, L.B. Bjostad & B.E. Hibbard, unpublished data).

Spatial distribution around roots

Early studies (Apple & Patel, 1963; Sechriest, 1969) documented that more rootworm larvae are found in maize roots than in soil early in the season. Among the best studies of rootworm distribution within the root system is that by Strnad and Bergman (1987b). They used a lactophenol and acid fuchsin stain (Goodey, 1937) to fix and visualize rootworm larvae in maize roots. Neonate WCR larvae were found predominantly on nodes three, four and five in the first year and nodes one, two and three in the second year. Third instar larvae were found mostly on nodes six, seven and eight in the first year and nodes six and seven in the second year. Significantly more first and second instars were oriented toward the root tip than the root base. Orientation within maize roots was not as clear for

third-instar larvae. First instars burrowed into root branches as small as 0.5 mm diameter. Most first instar larvae were in roots of 2 mm or less. In addition, significantly more first instar larvae than a random 33% were found in the distal third of the root than in the middle or proximal thirds. Later-instar larvae tended to leave the initial roots they were feeding on and move toward the newer nodes developing at the base of the stalk.

With commercialization of transgenic maize targeted toward WCR and NCR, have come studies evaluating rootworm spatial distribution under a variety of planting scenarios and seed combinations. WCR distribution and feeding behaviour differ between transgenic and isohline plants. Data from Hibbard *et al.* (2005) implied that both neonate and later-instar WCR larvae prefer nontransgenic roots to transgenic rootworm-resistant roots when a choice is possible. However, when damage to an infested, nontransgenic plant was high, WCR larvae apparently moved to neighboring transgenic rootworm-resistant plants and caused statistically significant damage. Clark *et al.* (2006) evaluated larval feeding in a medium that allowed direct observation. On rootworm-susceptible maize, neonate larvae initially began actively feeding. Some larvae that fed on the transgenic root exhibited no movement. Other larvae on the resistant maize moved continuously, sampling root hairs or root tissue but not actively feeding. These behaviours were dramatically different from those feeding on isohline maize. The continuously moving larvae had visibly empty guts, suggesting possible nonpreference for the resistant root. Altered feeding behaviour could be a mechanism that some larvae use to survive transgenic maize.

Adults

The chemical ecology of adult WCR in response to plant kairomones has been reviewed by Metcalf and Metcalf (1992). WCR–cucurbit interactions have been recently reviewed by Tallamy *et al.* (2005) and the specifics of pharmacophagy in these interactions on a more general scale have been reviewed by Gillespie *et al.* (2004).

Despite work on WCR phagostimulation related to the WCR–cucurbit interaction described in the above reviews, little similar work has focused on WCR relations with its main host, maize. Amino acids present in pollen lead to an increase in feeding of WCR on maize and squash pollen in comparison to sunflower and goldenrod in a no-choice experiment (Hollister & Mullin, 1999). They attributed these findings to the presence of a combination of specific amino acids. Lin and Mullin (1999) used a bioassay-driven fractionation to characterize phagostimulants in sunflower pollen. Lipids, including triglycerides, free fatty acids, phosphatidylethanolamines, phosphatidic acids and phosphatidylcholines, were highly phagostimulatory.

Although observed by many field entomologists previously, Prystupa *et al.* (1988) was first to formally demonstrate that WCR are attracted to maize silk. Abou-Fakhr *et al.* (1996) demonstrated that the senescing portions (the brown portion that protrudes from the tip of the ear) of maize silks elicited strong EAG responses from WCR adults, whereas the green portions (under the husk or very young, green silk beyond the husk) did not elicit a significant EAG response.

Hibbard *et al.* (1997) went on to isolate and identify the primary EAG-active components from brown maize silk as tridecan-2-one, (*E,E*)-3,5-octadien-2-one, (*E,Z*)-2,6-nonadienal, and (*E*)-2-nonenal. Hammack (1996) demonstrated that (*E*)-6,10-dimethyl-5,9-undecadien-2-one (geranylacetone) was highly attractive to NCR and also attractive to WCR. In a re-evaluation of the most EAG-active fractions from maize silk, Hibbard *et al.* (1997) found 6,10-dimethyl-5,9-undecadien-2-one as one of the smaller peaks present. The phenyl propenoids 2-phenethanol and benzyl alcohol were also present, but not in the most EAG-active fractions.

Significant questions about chemical ecology and physical cues affecting behaviour

Availability of identified and well-characterized host-plant feeding stimuli would enable revealing mechanistic studies of host recognition and acceptance that span the interface between behaviour and physiology. Investigation of these stimuli could enhance work to describe larval feeding behaviour and responses to the roots of *Bt*-transgenic and nontransgenic hybrids, as performed by Clark *et al.* (2006). Is altered larval feeding behaviour allowing some WCR to survive transgenic corn? If so, is this feeding behaviour heritable? Investigations of rootworm chemical ecology have shown promise as likely sources of *potential* management applications. Are there characteristics of WCR chemical ecology that prevent application of this knowledge?

Surprisingly little is known about any compounds that might influence WCR field ovipositional behaviour. Kirk (1979, 1981a, b) suggested that a crack or crevice and moisture are prerequisites for an oviposition site. Oviposition in varied crops and conditions by rotation-resistant WCR supports the view that moisture and physical cues are sufficient to stimulate oviposition [along with general cues such as carbon dioxide (Lance, 1992)]. Beyond Kirk's observations (1979, 1981a, b) and those of Gustin (1979), field characterization of WCR ovipositional stimuli and ovipositional behaviour is absent.

Conclusions

WCR are pests of historic importance with a legacy of resistance and behavioural plasticity. WCR behaviour is important and relevant to the past, the present and the future of rootworm management. The success of the most effective and environmentally benign management method (i.e. annual crop rotation) was firmly grounded on an understanding of behaviour and host relationships. Enthusiastic adoption of crop rotation, provided excellent rootworm management, but also selected for behavioural resistance to this cultural control. Understanding behavioural resistance would not have been possible without an understanding of behaviour (Spencer & Levine, 2008).

Although well-studied, significant gaps in our knowledge of WCR biology remain. A less than complete understanding of the status of alternate host use, larval and adult movement, mating behaviour, nutritional ecology, and chemical ecology may mean that assumptions critical to implementation of IPM and IRM are significantly off target. The trend toward increas-

ing adoption of transgenic crops for WCR management will place a premium on understanding WCR behaviour because the refuge components of IRM strategies (designed to promote sustainable deployment of transgenic hybrids) are grounded on assumptions about movement, mating and ovipositional behaviour. Transgenic maize hybrids will probably increasingly dominate the U.S. market for WCR management; revisiting our understanding of the pest's behaviour and ecology are needed to test whether assumptions about WCR activity and mate finding remain valid for WCR from transgenic maize.

Expansion of WCR populations into Europe has sparked a significant reconsideration of WCR ecology and behaviour. Conventional thinking and U.S.-derived management recommendations may not be directly transferable or workable in Europe. Researchers can look toward behaviour for clues to new mechanisms of management, or at the very least, for inspiration to refine current management.

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